AMENDMENTS TO THE SPECIFICATION

Replace paragraph [0052] of the original specification with the following paragraph:

[0052] A review of several methods for the isolation of oocysts can be found in Ryley ~~Ryler~~ *et al.* (*Parasitology* 73:311-326, 1976). In one method, described in U.S. Patent No. 3,147,186, oocysts are only crudely isolated following the addition of the oxidizing agent potassium dichromate. In this method, the moist droppings of host animals are directly mixed with an aqueous solution containing between one and four percent potassium chromate, preferably 2.5% or, less preferably, water, so that a suspension of thin consistency is obtained. The method indicates that a concentration of at least about one to four percent potassium chromate solution is necessary to obtain adequate oocyst sporulation. Larger insoluble debris, such as feathers and partially digested or undigested feed, is removed. Removal can be done conventionally by filtering the suspension through a mesh screen. The suspension is then allowed to stand for about five minutes to allow heavier coarser particles of debris that passed through the screen to settle to the bottom of the holding container. The supernatant liquid containing the oocysts is then removed. The sporulated oocysts are viable for up to about 18 hours.

Replace paragraph [0053] of the original specification with the following paragraph:

[0053] Another method for separating oocysts from droppings comprises flotation using solutions of sufficient specific gravity, typically having a specific gravity of about 1.2, so that oocysts float to the top of the suspension. Generally these solutions are made up of water to which a sugar (*e.g.* sucrose), $ZnSO_4$, or NaCl has been added to increase the specific gravity to the desired value. Useful solutions include solutions comprising 58% (w/v) sucrose, 37% (w/v) $ZnSO_4$ **[[ ]]** $\times$ $7H_2O$ and saturated NaCl solutions, which all have a specific gravity from about 1.09 to about 1.2. Other solutions which have a comparable specific gravity and are not harmful to the oocysts can also be used.

Replace paragraph [0056] of the original specification with the following paragraph:

[0056] Additional methods of oocyst isolation include, the use of glass bead columns (Ryley ~~Ryler~~ *et al.*, *Parasitology*, 73:311-326, 1976) and the bicarbonate ether method (Smith and Ruff, Poultry Sci. 54:2081-2086, 1975). In the glass column method, the aqueous suspension of fecal matter is added to a mixture of glass beads and a detergent, for example 5% Tween 80. The mixture is then applied to a column of glass beads and the oocysts are allowed to flow through while much of the undesired fecal matter is retained in the column. The effluent may then be concentrated by centrifugation.

Replace paragraph [0072] of the original specification with the following paragraph:

[0072] In one embodiment, the initial isolation is achieved by collecting manure from host animals, mixing the manure with domestic water, and then sieving. In one embodiment, the process begins with collected manure, *e.g.*, a batch of several hundred pounds, made into a aqueous slurry and processed to concentrate oocysts as a suspension in a relatively small volume of aqueous medium, *e.g.*, a several hundred pound ~~bound~~ batch of manure may ultimately yield about two liters of oocysts in an aqueous suspension (see Fig. 5A, "Challenge Suite", steps 1-3B). In this embodiment of the invention, sieving is by means of shaker screens, such as multiple tier shaker screens.

Replace paragraph [0090] of the original specification with the following paragraph:

[0090] In a preferred embodiment, the dense liquid is centrifuged at a temperature from about **[[**4 **]]** 4° C to about **[[**10 **]]** 10° C. The density of the liquid phase is then measured following the first centrifuge run using methods well known in

the art. If the density of the liquid is less than 1.09 g/ml, one should re-mix the liquid phase and the solid phase and add more high fructose corn syrup solution to obtain a density of 1.09 g/ml or greater. These steps can be repeated if necessary in order to obtain the highest yield of oocysts.

Replace paragraph [0097] of the original specification with the following paragraph:

[0097] In a preferred embodiment, the volume of the liquid fraction recovered from the flotation step is measured and a sample is taken to assess oocyst count. Sufficient domestic water is added to lower the density of the supernatant to less than about 1.04 g/ml. This allows the oocysts to sink. The density is measured following the addition of the domestic water using techniques well know in the arts. If the density is not less than about 1.04 g/ml and/or the oocysts have not sunk, additional domestic water is added until such density is reached and/or the oocysts sink. The oocyst suspension is then poured into centrifuge bottles and centrifuged for about 10 minutes at 1200 x g from about 4° C to about **[[**10 **]]** 10° C. The supernatant is tested for oocyst presence by counting using a microscope and hemocytometer and the supernatant is discarded if an acceptable amount of oocysts are counted in the supernatant. An acceptable amount of oocysts in the supernatant is from about 1% to about 5%, preferably about 2%, of the total oocysts loaded at the beginning of flotation step. More of the mixture from the flotation step is then poured on top of the solid fraction generated by centrifugation. While not necessarily being resuspended, the solid fraction is loosened somewhat, particularly by inverting the bottle a few times. The resuspended solid fraction suspension is then centrifuged as before, for about 10 minutes at 1200 x g from about **[[**4 **]]** 4° C to about **[[**10 **]]** 10° C, and the process is repeated until the flotation step mixture has all been centrifuged.

Replace paragraph [0098] of the original specification with the following paragraph:

[0098] When using the bottle centrifugation method, at this point, there should be several bottles, each with a solid fraction in the bottom. Note, however, with larger batch size the vessel or vessels vary with equipment that is of appropriate volume and recovery methods may be determined by one skilled in the art. The solid fractions in the centrifugation vessels are then resuspended by shaking them with a minimal amount of domestic water. The solid fractions are rinsed into one or two of the bottles and the bottles filled and balanced with water if necessary. These bottles are centrifuged one last time as before, for about 10 minutes at 1200 x g from about 4° C to about **[[**10 **]]** 10° C. The supernatant is then discarded. Any loose solid fractions that comes out with the supernatant can be ignored. The solid fraction is then resuspended in a minimal amount of domestic water and stored in a single bottle from about 2° C to about **[[**5 **]]** 5° C pending sporulation while freezing should be avoided.

Replace paragraph [0120] of the original specification with the following paragraph:

[0120] The pore size of the filter membrane should be small enough so that sporulated oocysts cannot enter the pores, but large enough to allow bacteria to pass through. In one embodiment, the filter has a pore size of approximately 10-microns. In yet another embodiment the filter has a pore size of approximately 5-microns. A preferred filtration unit is a ~~Consep~~ CONSEP® membrane unit manufactured by North Carolina SRT (available from North Carolina SRT, Inc., 221 James Jackson Ave., Cary, NC 27513). However, other filtration units may be used, such as those produced by Millipore (available from Millipore Corporation, 80 Ashby Road, Bedford, MA 01730). A preferred filter is the ~~Spectra/Mesh~~ SPECTRA/MESH® polyester filters (Spectrum Laboratories, Inc., Rancho Dominguez, CA; cat no: 146524). Tangential filtration units such as an ~~OPTISEP CL, OPTISEP, or CONSEP~~ OPTISEP CL®, OPTISEP®, or CONSEP® filtration unit may be used, also available from North Carolina SRT. Throughput can be increased by utilizing a larger scale filtration unit. One skilled in the art will recognize that the type of filtration unit needed depends on the volume of the sporulated oocyst suspension. In one embodiment, an ~~OPTISEP CL~~ OPTISEP CL®

filtration unit is used to run about a 1L sporulated oocyst suspension. In another embodiment, a ~~CONSEP~~ CONSEP® filtration unit is used to run about a 10L sporulated oocyst suspension.

Replace paragraph [0122] of the original specification with the following paragraph:

[0122] In a further embodiment, the inlet and the outlet tubing for the tangential flow unit are placed into a vessel containing the sporulated oocysts while the permeate tubing is placed in a separate vessel. The pump, for example, a diaphragm pump, is then started to begin filtration. A preferred flow rate is about 1 LPM per 160 cm^2. The pump rate may also be expressed in terms of lineal velocity. Lineal velocities may be between 20 and 50 centimeters per second. A preferred lineal velocity when using a ~~CONSEP~~ CONSEP® filtration unit is 28 centimeters per second. The pump may be kept running to maintain the flow rate throughout the process. The permeate is sampled and, using a glass slide, observed for sporulated oocysts. The optical density of the permeate sample is also measured using a spectrophotometer at 600 nm (OD_{600}). Circulation of retentate over the filter medium is continued if the concentration of oocysts in the permeate has not increased to or exceed a maximum tolerable level. An acceptable concentration is from about not more than 5% of the total sporulated oocysts loaded into the filtration unit. If the sporulated oocysts concentration measures to or above the maximum acceptable level, filtration is stopped. The permeate is recycled and mixed with the retentate and filtration is resumed. Filtration is stopped when the measured OD_{600} is about less than about 0.5 at a lineal velocity of about 28 centimeters per second. However, filtration may be stopped when the measured OD_{600} is about 0.6, again at a lineal velocity of about 28 centimeters per second. Once the desired OD is reached, oocysts from the membranes and the tubing are transferred to the retentate vessel and any oocysts remaining in the membranes and tubing are then flushed with water into the retentate vessel.

Replace paragraph [0131] of the original specification with the following paragraph:

[0131] While the hypochlorite-rich permeate leaves the filtration unit, sterile water is added to replenish the volume. Filtration is continued and water is added as needed to control the percent solids during filtration. A sample is then taken from the permeate to determine total chlorine level. Chlorine level can be detected using ~~CHEMetrics Vacuette Kit,~~ chlorine detection kits, such as those available from CHEMetrics, Inc., Route 28, Calverton, VA, 20138. The total level of chlorine should is reduced to less than about 1 ppm **[[**by**]]**. When the permeate contains less than about 1 ppm of the chlorine the retentate, which contains the oocysts, also contains less than about 1 ppm. Once the desired level of chlorine is reached, filtration may be continued without adding more water to reduce the overall volume of the sporulated oocysts suspension.

Replace paragraph [0134] of the original specification with the following paragraph:

[0134] For the purposes of the present invention, sporulated oocysts and compositions containing sporulated oocysts are considered sterile if samples of liquids containing the oocysts do not have detectable amounts of live bacteria, IBD virus or CAV virus. Detection of live bacteria can be accomplished by any method known in the art. For example, bacteria can be detected by incubation on bacterial agar plates at 35-37° C for 18 to 24 hours. One preferred method of testing is set forth by the USDA in 9 C.F.R. 113.27 (1999), hereby incorporated in its entirety by reference. Briefly, to test for bacterial contamination, a sample of the preparation of the present invention can be innoculated into soybean casein digest medium and incubated at 30 to 35° C for 14 days. To test for fungal contamination, a sample of the preparation of the present invention can be innoculated into soybean casein digest medium and incubated at 20 to 25° C for 14 days. After the incubation period, the vessels can be examined macroscopically for microbial growth. If growth cannot be determined reliably by visual examination, the judgment can be confirmed by microscopic examination.

Replace paragraph [0135] of the original specification with the following paragraph:

[0135] Detection of IBDV virus or CAV virus can be by any method known in the art. A non-limiting example of IBDV and CAV detection is by the methods set forth by the USDA in 9 C.F.R. 113.47 (1999), herein incorporated in its entirety by reference. Briefly, to test for CAV, MSB-1 cells from the Maine Biological Laboratories, Waterville, ME are used as the indicator cell line for Chicken Anemia Virus. MSB-1 cells are a lymphoblastoid cell line from a Marek's disease lymphoma that show cytopathic effect when infected with Chicken Anemia Virus. Cells are maintained in a medium, such as OPTI-MEM® ~~Opti-MEM®~~ (Life Technologies, Gaithersburg, MD) or other suitable media at 41° C for at least 24 days prior to testing. Cells are subcultured 10-12 times during the maintenance period with all but the last subculture resulting in a monolayer of at least 75 cm^2. The last subculture is at least 6 cm^2.

Replace paragraph [0167] of the original specification with the following paragraph:

[0167] To the oocysts obtained as described in Example 1, was added enough of a 5.25% sodium hypochlorite solution ~~(CLOROX)~~ to obtain a final concentration 0.05 wt% sodium hypochlorite. This oocyst/sodium hypochlorite mixture was added to a 10 liter fermentor set at 28 + 1°C and an agitation rate of 200 RPM. Oxygen was provided by portable oxygen cylinders and bubbled through the mixture at a rate sufficient to obtain a percent saturation of dissolved oxygen value of at least 50% of saturation of dissolved oxygen. Oxygen flow was adjusted so as not to cause foaming of the mixture. The oocysts were maintained under these conditions for about 72 hours. During sporulation, dissolved oxygen and pH were constantly monitored. It was observed that beginning at approximately 12 hours into the sporulation process there was a decrease in the percent saturation of dissolved oxygen (increased oxygen consumption) followed by an increase in pH and a return of dissolved oxygen to previous levels (**Figure 1**). In some, but not all cases, the increase in pH was preceded by a decrease in pH at about

the same time as the decrease in the percent saturation of dissolved oxygen (**Figure 2**). These changes in dissolved oxygen and pH were found to be reliable indicators of sporulation. Examination of oocysts following these change showed a high degree of sporulation. In contrast when these changes were not observed, the sporulation rate was dramatically reduced from approximately 90% to approximately 10%. Although sporulation was complete at approximately 24 to 36 hours, the incubation was continued for another 36 to 48 hours to provide a more stable sporulated oocyst population.

Replace paragraph [0171] of the original specification with the following paragraph:

[0171] Following sporulation, the sporulated oocysts were concentrated by tangential flow filtration. To begin, the integrity of the filter membrane was visually observed prior to assembling the ~~CONSEP~~ CONSEP® tangential flow filtration system. The filter unit was then assembled according to the appropriate standard operating procedure ("SOP") as provided in the manufacture's manual. After the system was assembled about 2 to 4 liters of cold domestic water was run through the system to check for leaks. If leaks were found, the system would be disassembled and reassembled after checking for the source of the leakage. Particular attention was paid to inspecting the gaskets to assure lack of damage to the gaskets and for proper seating.